U.S. Application No. Unknown	International Application No. PCT/EP00/03588	10/089040 Attorney Docket No. WWELL53.001APC

Date: October 23, 2001

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 USC 371

International Application No.:	PCT/EP00/03588
International Filing Date:	April 20, 2000
Priority Date Claimed.	April 23, 1999
Title of Invention:	USE OF COXSACKIE VIRUSES FOR IMPROVING TRANSFECTION OF CELLS
Applicant(s) for DO/EO/US:	KUPPER, Jan-Heiner, KANDOLF, Reinhard and SELINKA, Hans-Christoph

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. (X) This is a **FIRST** submission of items concerning a filing under 35 USC 371.

2. () This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 USC 371.

3. (X) This express request to begin national examination procedures (35 USC 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 USC 371(b) and PCT Articles 22 and 39(1).

4. (X) A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

5. (X) A copy of the International Application as filed (35 USC 371(c)(2))

   a) () is transmitted herewith (required only if not transmitted by the International Bureau)
   b) (X) has been transmitted by the International Bureau.
   c) () a copy of Form PCT/1B/308 is enclosed.
   d) () is not required, as the application was filed in the United States Receiving Office (RO/US).

6. (X) A translation of the International Application into English and two (2) pages of drawings (35 USC 371(c)(2)).

7. (X) Amendments to the claims of the International Application under PCT Article 19 (35 USC 371(c)(3))

   a) () are transmitted herewith (required only if not transmitted by the International Bureau).
   b) () have been transmitted by the International Bureau.
   c) () have not been made; however, the time limit for making such amendments has NOT expired.
   d) (X) have not been made and will not be made.

8. () A translation of the amendments to the claims under PCT Article 19 (35 USC 371(c)(3)).

9. () An oath or declaration of the inventor(s) (35 USC 371(c)(4)).

10. (X) A copy of the International Preliminary Examination Report with any annexes thereto, such as any amendments made under PCT Article 34.

11. () A translation of the annexes, such as any amendments made under PCT Article 34, to the International Preliminary Examination Report under PCT Article 36 (35 USC 371(c)(5)).

12. (X) An Information Disclosure Statement under 37 CFR 1.97 and 1.98 with ten (10) references.

13. () An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

14. (X) A FIRST preliminary amendment.
() A SECOND or SUBSEQUENT preliminary amendment.

15. () A substitute specification.

16. () A power of attorney and/or address letter.

17. (X) International Application as published.

18. (X) Paper Copy of the Sequence Listing in 3 pages and CRF (diskette).

19. (X) Sequence Submission Statement.

20. (X) International Search Report.

21. () The present application qualifies for small entity status under 37 C.F.R. § 1.27.

22. (X) A return prepaid postcard.

23. (X) The following fees are submitted: **FEES**

	BASIC FEE			$890
CLAIMS	**NUMBER FILED**	**NUMBER EXTRA**	**RATE**	
Total Claims	45 - 20 =	25 ×	$18	$450
Independent Claims	3 - 3 =	0 ×	$84	$0
Multiple dependent claims(s) (if applicable)			$280	$0
	TOTAL OF ABOVE CALCULATIONS		$1340	
Reduction by 1/2 for filing by small entity (if applicable). Verified Small Entity statement must also be filed. (NOTE 37 CFR 1.9, 1.27, 1.28)			$	
	TOTAL NATIONAL FEE			$1340
	TOTAL FEES ENCLOSED			$1340
		amount to be refunded:		$
		amount to be charged:		$

24. (X) The fee for later submission of the signed oath or declaration set forth in 37 CFR 1.492(e) will be paid upon submission of the declaration.

25. (X) A check in the amount of $1340 to cover the above fees is enclosed.

26. () Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40 per property.

27. (X) The Commissioner is hereby authorized to charge only those additional fees which may be required, now or in the future, to avoid abandonment of the application, or credit any overpayment to Deposit Account No. 11-1410.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Mark R. Benedict
Reg. No. 44,531
Customer No. 20,995

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101901

WWELL53.001APC

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Küpper, et al.	Group Art Unit Unknown
Appl. No.	:	Unknown	
Filed	:	Herewith	
For	:	USE OF COXSACKIE VIRUSES FOR IMPROVING TRANSFECTION OF CELLS	
Examiner	:	Unknown	

I hereby certify that this correspondence and all marked attachments are being deposited with the United States Postal Service as first-class mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231, on

October 23, 2001
(Date)

Mark R. Benedict, Reg. No. 44,531

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Prior to examination on the merits, please amend the above-captioned patent application as follows:

IN THE SPECIFICATION:

Please amend the specification as follows:

On page 1 following the Title of the Invention, please insert the following paragraph:

Cross-Reference to Related Applications

This application is the U.S. National Phase under 35 U.S.C. § 371 of International Application No. PCT/EP00/03588, which was published in German and claims priority to German patent application number 19918446.1, filed April 23, 1999. This German patent application is herein incorporated by reference.

Appl. No. : **Unknown**
Filed : **Herewith**

On page 1, line 3, before the paragraph beginning "The present invention...", please insert the following heading:

Field of the Invention

On page 1, line 7, before the paragraph beginning "Coxsackie viruses belong...", please insert the following heading:

Background of the Invention

On page 6, line 4, before the paragraph beginning "In view of the above...", please insert the following heading:

Summary of the Invention

On page 7, line 18, before the paragraph beginning "The inventors of the present invention...", please insert the following heading:

Detailed Description of the Invention

On page 28, line 1, please omit the heading, "Claims", and replace it with the following heading:

What is claimed is:

IN THE ABSTRACT:

Please insert the Abstract attached hereto, following the pages entitled VERSION WITH MARKINGS TO SHOW CHANGES MADE, as page 33 of the application as filed.

IN THE CLAIMS:

Please cancel Claims 11 and 12.

Please amend the claims as follows:

1. Non-infectious particles derived from the Coxsackie virus, which are suitable to act endosomolytically within cells transfected with said particles.

2. Non-infectious particles derived from the Coxsackie virus, which are suitable to enhance endocytosis within cells transfected with said particles.

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Filed : **Herewith**

3. The non-infectious particles of Claim 1, selected from the group consisting of:

a) empty CV capsid particles and CVB capsid particles;

b) CV particles and CVB-A particles, generated by cleaving off virus protein 4 (VP4);

c) CV particles and CVB provirus particles, in which the virus proteins 2 (VP2) and 4 (VP4) are still fused;

d) modified forms of particles from a), b), and c); and

e) CV particles, CVB particles,]and CVB3 particles, that are replication-incompetent by genetic modification.

4. The non-infectious particles of claim 1, wherein said particles comprise peptides which are less than 30 amino acids and are selected from group consisting of:

a) Peptides with the amino acid sequences SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 5, SEQ ID No. 6, SEQ ID No. 7, SEQ ID No. 8, SEQ ID No. 9, and SEQ ID No. 10; and

b) Peptides which in comparison with peptides from a) contain an amino acid deletion or substitution.

5. A method for generating the particle of claim 3, comprising the steps of:

- Isolating native Coxsackie virus particles;
- Heating the isolated Coxsackie virus particles for 5-20 min to over 45°C.

6. A method for generating the particle of claim 3, comprising the steps of:

- Providing a matrix of coupled Coxsackie virus receptor;
- Loading the matrix with native Coxsackie virus particles; and
- Eluting the matrix.

7. A method for transfection of cells with a polyanionwherein at least one particle of claim 1 is used in order to enhance transfection.

8. The method of claim 7, wherein prior to transfection a complex comprising said polyanion, peptide and cationic lipid is formed.

9. A method for formulating complexes for the transfer of a polyanion into cells in which method the polyanion is incubated with at least one particle of claim 1.

10. The method of claim 9, wherein the peptide is pre-incubated with a cationic lipid prior to the incubation with said polyanion.

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Filed : Herewith

13. A replicable expression vector comprising a gene sequence encoding a particle according to claim 1 in an expressible manner.

14. A DNA isolate comprising a DNA sequence encoding a particle according to claim 1.

15. A kit for formulating transfectable complexes comprising at least one particle according to claim 1.

16. A therapeutic composition, comprising at least one particle according to claim 1.

17. A vector plasmid, comprising at least one DNA sequence encoding a genetically modified CV genome, in which parts of the coding sequence are exchanged or modified, so that the virus genome is not infectious, and comprising a promoter upstream of said DNA sequence.

18. A helper construct for complementing the parts of the coding sequence of said virus genome that have been modified or exchanged in the vector plasmid of claim 17.

19. The helper construct of claim 18, comprising a helper plasmid encoding at least one of said exchanged or modified parts in a translatable manner.

20. The helper construct of claim 18, comprising a viral vector encoding at least one of said exchanged or modified parts in a translatable manner.

21. A helper cell comprising helper DNA encoding at least one of said exchanged or modified parts according to Claim 17.

22. A method for generating a CV particle that is replication-incompetent by genetic modification, comprising the steps of:

- Transfecting host cells with the vector plasmid of claim 17; and
- Complementing said exchanged or modified parts in the host cell with a helper construct.

23. The method of claim 22, wherein said host cell is the helper cell of claim 21.

Please add the following claims:

24. The non-infectious particles of Claim 2, selected from the group consisting of:

a) empty CV capsid particles and CVB capsid particles;

b) CV particles and CVB-A particles, generated by cleaving off virus protein 4 (VP4);

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Filed : **Herewith**

c) CV particles and CVB provirus particles, in which the virus proteins 2 (VP2) and 4 (VP4) are still fused;

d) modified forms of particles from a), b), or c); and

e) CV particles, CVB particles, and CVB3 particles, that are replication-incompetent by genetic modification.

25. The non-infectious particles of claim 1, wherein said particles comprise peptides which are less than 30 amino acids and are selected from group consisting of:

a) Peptides with the amino acid sequences SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 5, SEQ ID No. 6, SEQ ID No. 7, SEQ ID No. 8, SEQ ID No. 9, and SEQ ID No. 10; and

b) Peptides which in comparison with peptides from a) contain an amino acid deletion or substitution.

26. A method for generating the particle of claim 24, comprising the steps of:

- Isolating native Coxsackie virus particles;

- Heating the isolated Coxsackie virus particles for 5-20 min to over 45°C

27. A method for generating the particle of claim 24, comprising the steps of:

- Providing a matrix of coupled Coxsackie virus receptor;

-Loading the matrix with native Coxsackie virus particles; and

- Eluting the matrix.

28. A method for therapy, diagnostics or prophylaxis of cardiovascular diseases, comprising the step of transfection of cells involved in said diseases, and wherein said transfection step comprises transfecting said cells with at least one particle of Claim 1.

29. An agent for improving transfection of cells, comprising at least one particle of Claim 1.

30. The method according to claim 5, wherein said Coxsackie virus particles are heated for approximately 10 min to over 45°C.

31. A method according to claim 5, wherein said Coxsackie virus particles are heated to approximately 51°C.

32. The method according to claim 7 wherein said cells are cardiac myocytes.

33. The method according to claim 7 wherein said polyanion is a therapeutic gene sequence.

34. The method according to claim 9 wherein said cells are cardiac myocytes.

35. The method according to claim 9 wherein said polyanion is a therapeutic gene sequence.

36. The method according to claim 26, wherein said Coxsackie virus particles are heated for approximately 10 min to over 45°C.

37. A method according to claim 26, wherein said Coxsackie virus particles are heated to approximately 51°C.

38. A method for transfection of cells with a polyanion wherein at least one particle of claim 2 is used in order to enhance transfection.

39. The method of claim 38, wherein prior to transfection a complex comprising said polyanion, peptide and cationic lipid is formed.

40. A method for formulating complexes for the transfer of a polyanion into cells in which method the polyanion is incubated with at least one particle of claim 2.

41. The method of claim 40, wherein the peptide is pre-incubated with a cationic lipid prior to the incubation with said polyanion.

42. A method for therapy, diagnostics or prophylaxis of cardiovascular diseases, comprising the step of transfection of cells involved in said diseases, and wherein said transfection step comprises transfecting said cells with at least one particle of Claim 2.

43. An agent for improving transfection of cells, comprising at least one particle of Claim 2.

44. A replicable expression vector comprising a gene sequence encoding a particle according to Claim 2 in an expressible manner.

45. A DNA isolate comprising a DNA sequence encoding a particle according to claim 2.

46. A kit for formulating transfectable complexes comprising at least one particle according to Claim 2.

47. A therapeutic composition, comprising at least one particle according to Claim 2.

REMARKS

Claims 1-10 and 13-23 were amended, Claims 11-12 were cancelled, and Claims 24-47 were added to more clearly claim the invention and place them in accordance with conventional practices of the United States Patent and Trademark Office. No new matter has been added.

Appl. No. : **Unknown**
Filed : **Herewith**

Changes to the claims can be seen on separate pages entitled VERSION WITH MARKINGS TO SHOW CHANGES MADE following the signature page. Deletions are in **[bold and brackets]**, and insertions are underlined.

Conclusion

Should any questions exist which would delay prosecution of the application filed herewith, the Examiner is respectfully invited to contact the under-signed attorney at the telephone number below.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

Dated: 10/23/01

By: _____

Mark R. Benedict
Registration No. 44,531
Attorney of Record
620 Newport Center Drive
Sixteenth Floor
Newport Beach, CA 92660
(949) 721-6323

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Appl. No. : **Unknown**
Filed : **Herewith**

VERSION WITH MARKINGS TO SHOW CHANGES MADE
Deletions are in **[bold and brackets]**, and insertions are underlined.

IN THE SPECIFICATION:

Please amend the specification as follows:

On page 1 following the Title of the Invention, please insert the following paragraph:

Cross-Reference to Related Applications

This application is the U.S. National Phase under 35 U.S.C. § 371 of International Application No. PCT/EP00/03588, which was published in German and claims priority to German patent application number 19918446.1, filed April 23, 1999. This German patent application is herein incorporated by reference.

On page 1, line 3, before the paragraph beginning "The present invention...", please insert the following heading:

Field of the Invention

On page 1, line 7, before the paragraph beginning "Coxsackie viruses belong...", please insert the following heading:

Background of the Invention

On page 6, line 4, before the paragraph beginning "In view of the above...", please insert the following heading:

Summary of the Invention

On page 7, line 18, before the paragraph beginning "The inventors of the present invention...", please insert the following heading:

Detailed Description of the Invention

On page 28, line 1, please omit the heading, "Claims", and replace it with the following heading:

What is claimed is:

Appl. No. : Unknown
Filed : Herewith

IN THE CLAIMS:

Please cancel Claims 11 and 12.

Please amend the claims as follows:

1. Non-infectious particles**[or peptides]** derived from the Coxsackie virus**[, preferably derived from CVB, more preferably derived from CVB3]**, which are suitable to act endosomolytically within **[the scope of a transfection of]**cells transfected with said particles.

2. Non-infectious particles**[or peptides]** derived from the Coxsackie virus**[, preferably derived from CVB, more preferably derived from CVB3]**, which are suitable to enhance endocytosis within [the scope of a transfection of]cells transfected with said particles.

3. The non-infectious p**[P]**articles of Claim 1**[or 2]**, selected from the group consisting of:

a) empty CV capsid particles and**[, preferably]** CVB capsid particles;

b) CV particles and**[, preferably]** CVB-A particles, generated by cleaving off virus protein 4 (VP4);

c) CV particles and**[, preferably]** CVB provirus particles, in which the virus proteins 2 (VP2) and 4 (VP4) are still fused;

d) modified forms of particles from a), b), **[or]**and c); and

e) CV particles, **[preferably]** CVB particles,**[more preferably]**and CVB3 particles, that are replication-incompetent by genetic modification.

4. The non-infectious particles**[A peptide]** of claim**[s]** 1**[or 2, preferably comprising]**, wherein said particles comprise peptides which are less than 30 amino acids and are **[being]** selected from group consisting of:

a) Peptides with the amino acid sequences SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 5, SEQ ID No. 6, SEQ ID No. 7, SEQ ID No. 8, SEQ ID No. 9, and SEQ ID No. 10**[from the sequence listing]**; and

b) Peptides**[, in]**which in comparison with peptides from a) contain an amino acid deletion or substitution**[one or more of the amino acids are missing or exchanged against another; and**

c) Peptides or polypeptides which comprise the amino acid sequence of one of the peptides from a) or b)].

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Filed : **Herewith**

5. A method for generating **[a]**the particle of claim 3, comprising the steps of:

- Isolating native Coxsackie virus particles;

- Heating the isolated Coxsackie virus particles for 5-20 min**[, preferably approximately 10 min]** to over 45°C**[, preferably approximately 51°C]**.

6. A method for generating **[a]**the particle of claim 3, comprising the steps of:

- Providing a matrix of coupled Coxsackie virus receptor;

- Loading the matrix with native Coxsackie virus particles; and

- Eluting the matrix.

7. A method for transfection of cells**[, preferably of cardiac myocytes,]** with a polyanion**[, preferably a therapeutic gene sequence, in which method]**wherein at least one particle **[or peptide]** of **[any of]** claim**[s]** 1 **[through 4]** is used in order to enhance transfection.

8. The method of claim 7, **[characterized in that]**wherein prior to transfection a complex comprising said polyanion, peptide and cationic lipid**[, preferably DOTMA,]** is formed.

9. A method for formulating complexes for the transfer of a polyanion**[, preferably a therapeutic gene sequence,]** into cells**[, preferably into cardiac myocytes,]** in which method the polyanion is incubated with at least one particle**[or peptide of one]** of claim**[s]** 1**[through 4]**.

10. The method of claim 9, **[characterized in that]**wherein the peptide is pre-incubated with a cationic lipid**[, preferably with DOTMA,]** prior to the incubation with said polyanion.

13. A replicable expression vector comprising a gene sequence encoding**[a peptide or]** a particle according to **[any of]**claim**[s]** 1**[through 4]** in an expressible manner.

14. A DNA isolate comprising a DNA sequence encoding a particle**[or a peptide]** according to **[any of]**claim**[s]** 1**[through 4]**.

15. A kit for formulating transfectable complexes**[, in particular for gene therapy,]** comprising at least one particle**[or one peptide]** according to **[any of]**claim**[s]** 1**[through 4]**.

16. A therapeutic composition, comprising at least one particle**[or a peptide]** according to**[any of]** claim**[s]** 1**[through 4]**.

17. A vector plasmid, comprising at least one DNA sequence encoding a genetically modified CV genome**[, preferably a CVB genome, more preferably a CVB3 genome]**, in

Appl. No. : **Unknown**
Filed : **Herewith**

which parts of the coding sequence are exchanged or modified, so that the virus genome is not infectious, and comprising a promoter upstream of said DNA sequence.

18. A helper construct for complementing the parts of the coding sequence of said virus genome that have been modified or exchanged in the vector plasmid of claim 17.

19. The helper construct of claim 18, comprising**[characterized in that it has]** a helper plasmid encoding at least one of said exchanged or modified parts in a translatable manner.

20. The helper construct of claim 18, comprising**[characterized in that it is]** a viral vector encoding at least one of said exchanged or modified parts in a translatable manner.

21. **[The helper construct of claim 18, characterized in that it is a]**A helper cell comprising**[that is stably transfected with]** helper DNA encoding at least one of said exchanged or modified parts according to Claim 17.

22. A method for generating a CV particle**[said particle of claim 3e)]**that is replication-incompetent by genetic modification, comprising the steps of:

- Transfecting host cells with the vector plasmid of claim 17; and

- Complementing said exchanged or modified parts in the host cell with a**[by the]**helper construct**[according to any of claims 18 through 20]**.

23. The method of claim 22, **[characterized in that]**wherein said host cell is the helper cell of claim 21.

IN THE ABSTRACT:

Please insert the Abstract attached hereto as page 33 of the application as filed.

Abstract

The present invention is related to the use of Coxsackie viruses (CV) for improving transfection of cells.

WWELL53.001APC

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Küpper, et al.	Group Art Unit Unknown
Appl. No.	:	Unknown	
Filed	:	Herewith	
For	:	USE OF COXSACKIE VIRUSES FOR IMPROVING TRANSFECTION OF CELLS	
Examiner	:	Unknown	

SEQUENCE SUBMISSION STATEMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

A copy of the Sequence Listing in computer readable form as required by 37 C.F.R. § 1.821(e) is submitted herewith.

As required by 37 C.F.R. § 1.821(f), I hereby declare that the information recorded on the enclosed disk is identical to the printed Sequence Listing in the application filed herewith.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

Dated: 10/23/01

By: Mark R. Benedict

Mark R. Benedict
Registration No. 44,531
Attorney of Record
620 Newport Center Drive
Sixteenth Floor
Newport Beach, CA 92660

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PCT10

RAW SEQUENCE LISTING
PATENT APPLICATION: US/10/009,040

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TIME: 12:30:24

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Kandolf, Reinhard
Selinka, Hans-Christoph
Eberhard-Karls-Universitat Tubingen Universitatsklinikum
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<141> CURRENT FILING DATE: 2001-10-23
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<151> PRIOR FILING DATE: 1999-04-23
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VERIFICATION SUMMARY
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TIME: 12:30:25

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Output Set: N:\CRF3\01142002\J009040.raw

L:14 M:270 C: Current Application Number differs, Replaced Current Application Number

SEQUENCE LISTING

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Kandolf, Reinhard
Selinka, Hans-Christoph
Eberhard-Karls-Universitat Tubingen Universitatsklinikum

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Use of Coxsackie Viruses for improving Transfection of Cells

The present invention is generally related to the use of Coxsackie viruses (in the following: CV) for improving transfection of cells.

Coxsackie viruses belong to the family of Picornaviridae and are classified in subgroup A comprising serotypes 1-22 and 24 and subgroup B comprising serotypes 1-6. While it is generally referred to Coxsackie viruses in the scope of the invention, the invention is particularly related to Coxsackie viruses of subgroup B, preferably of serotype B3.

In the scope of the present application, transfection does not only implicate introducing DNA or RNA into cells, but also introducing peptides into cells. The transfection can, thus, be used for therapeutic, diagnostic or prophylactic purposes, in particular for gene therapy.

The present invention gives particular attention to diagnosis, treatment and prevention of cardiac diseases which become more and more important in particular in industrial nations. As different genes have already been identified for cardiac diseases and it is generally expected that a plurality of pathological genes that may cause cardiac diseases will be identified in the years to come, development of cardiac-muscle-specific gene transfer systems for selective modulation of endogenous gene activity of cardiac myocytes for the future treatment of a plurality of congenital and acquired cardiac muscle diseases is of high clinical importance. Ideal vector systems for controlled modulation of endogenous gene activities of cardiac myocytes are, however, not available by now.

It is true that for the transfection of target cells of the cardiovascular system, different techniques have already been described, but all these show specific disadvantages. In preclinical studies for somatic gene therapy of cardiovascular diseases, in particular replication defect recombinant adeno viruses were tried, by means of which a sufficient transfection efficiency is obtained (Barr et al., Gene Therapy 1, 1994, 51). In contrast to recombinant adeno viruses, application of retroviral constructs (Nabel EG et al., Science 249, 1990, 1285) and of lipid/DNA/complexes (Nabel EG et al, Science 244, 1989, 1342) is limited by low transfection efficiency of gene

transfer. In spite of the advantages of the adeno virus mediated gene transfer with reference to high gene expression, the disadvantages of this method are the complementation of replication inactive constructs, as known from literature, the multiorganotrophism of adeno viruses and the possible induction of a T-cell mediated immune response.

Felgner et al., PNAS, vol. 84, pages 7413-7417, 1987, describe a transfection of DNA, called lipofection, into cell culture cells, in which a liposome formulation of the cationic lipid N-[1-(2,3-dioleyloxy)propyl]-n,n,n-trimethylammoniumchloride (DOTMA) and Dioleoyl-phosphotidylethanolamine (PtdEtn) is used. The DNA to be transfected interacts spontaneously with the liposome formulation, and lipid-DNA-complexes are formed. The fusion of the complex with cell culture cells results in an efficient uptake and expression of the DNA.

This liposome formulation having the trade name LIPOFECTIN® is merchandised by GIBCO/BRL. The production information supplied therewith, in which PtdEtn is designated as DOPE, discloses detailed instructions for carrying out the lipofection.

Meanwhile, lipofection is an accepted method to introduce recombinant DNA into cells and to express same in the cells. Since lipofection is superior to viral vector systems for what concerns safety, it is increasingly attempted to use this technique also for gene therapy of metabolism or tumor diseases. Efficiency, however, is low in most of the applications, in particular for primary cultures or in-vivo-applications the known liposome systems are not well suitable up to now.

A further attempt starts from the idea that many viruses are taken up by cellular receptors, reach cytoplasmatic endosomes on the natural way and start their replication cycle from there, after breaking up of the endosomes. Cotten et al., PNAS vol. 89, pages 6094-6098, 1992, make use of this endosomolytic effect and use replication incompetent adeno viruses as an endosomolytic agent together with transferrin as a ligand for the target cell receptors.

Wagner et al., PNAS vol. 89, pages 7934-7938 show that peptide fragments of an influenza virus act endosomolytically. As a synthetic, virus-like gene transfer vehicle they use DNA complexes containing polylysine for packaging the DNA, polylysine-modified transferrin as a ligand for the cell surface receptors and polylysine-bound influenza peptide fragments as the endosomolytic agent.

The methods described comprise, on the one hand, great efforts and, thus, are not suitable for standard applications. Moreover, for the last two methods described above, very large amounts of peptides are required, which is disadvantageous due to the high immunogenity for in-vivo-applications and is, moreover, also unsuitable for in-vitro-applications due to the expensive manufacturing.

Kern et al. describe another approach in "Coxsackie virus-reinforced endosomolytic Gene Transfer in contractile Cardiomyocytes", Verh. Dtsch. Ges. Path. volume 81, page 611, 1997. Assuming that the Coxsackie virus comprises a tropism towards the heart not understood up to now, they use during lipofection CVB3 particles, made replication incompetent by UV radiation,

for gene transfer in cardiomyocytes. CVB3, a Coxsackie virus of subgroup B with serotype 3, is a picorna virus containing a single-stranded RNA-genome positive in polarity and a genome size of only 7.4 kb. In comparison, adeno viruses have a genome size of 48 kb.

The method of generating replication incompetent CVB3 particles by UV radiation is, however, not safe enough. According to the finding of the inventors of the present application, it can namely not be excluded that some virus particles survive and then begin a productive infection in the target cells.

Kandolf and Hofschneider, PNAS, volume 82, pages 4818/4822, 1985, describe a CVB3 variant with distinct tropism towards the heart. The complete nucleotide sequence of the cDNA of this infectious CVB3 variant is described in Klump et al., Journal of Virology, 1990, pages 1573-1783. It is reported that the cDNA-derived virus comprises the same tropism and the same plaque morphology as the wild type.

Kern et al. (loc. cit.) describe that the inactivated CVB3 imparts a reinforcement of the transfection mediated by the liposome formulation DOTAP (Böhringer). The maximum transfection efficiency is reported for the use of 10 pfu (plaque forming units) of inactivated CVB3 per cell, it was up to 6 fold higher for the reporter gene β-galactosidase than for transfection of the reporter gene construct containing DOTAP only.

Although it is known from the publication by Kern et al. that the CVB3-reinforced DOTAP-mediated lipofection leads to a faster escape of transfected DNA from the endosomes into the

cytosol, the efficiency described as well as the high use of CVB3 is not satisfying for immunological reasons.

In view of the above, it is an object of the present invention to provide enhancement of the transfection efficiency of cells, wherein the method is to be cost-effective, safe and immunologically harmless at the same time.

According to the invention, this object is achieved by non-infectious particles or peptides derived from the Coxsackie virus, preferably derived from CVB, which are suitable to act endosomolytically or to enhance endocytosis, within the scope of transfection of cells.

The particles are hereby selected from the group

a) empty CV capsid particles, preferably CVB capsid particles;

b) CV particles, preferably CVB-A particles formed by cleaving off virus protein 4;

c) CV particles, preferably CVB provirus particles, in which the virus proteins 2 and 4 are still fused;

d) modified forms of particles from a), b) or c); and

e) CV particles preferably CVB particles, more preferably CVB3 particles, that are replication-incompetent by genetic modification.

The peptide which comprises preferably less than 30 amino acids is hereby selected from the group:

a) Peptides having the amino acid sequences SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 5, SEQ ID No. 6, SEQ ID No. 7, SEQ ID No. 8, SEQ ID No. 9, SEQ ID No. 10 from the enclosed sequence listing;

b) Peptides, in which in comparison with peptides from a) one or more of the amino acids are missing or exchanged against another; and

c) Peptides or polypeptides which comprise the amino acid sequence of one of the peptides from a) or b).

The object underlying the invention is in that way completely achieved.

The inventors of the present invention have recognized, namely, that particles or peptides derived from the CV, preferably CVB, more preferably from the CVB3, can be used for gene transfer, as they act endosomolytically and/or reinforce the endocytosis. On the one hand, the method of transfection becomes very cheap by use of these particles, since very high yields are produced, a very simple purification is possible, and very small peptides/particles are used which contain a considerably lower number of immunogenous epitopes as adeno or influenza viruses. The inventors were able to show that optimal biological effects arise for much lesser peptide amounts than for adeno viruses, the higher biological effect, further, going together with a decrease of immunogenity.

Another advantage of the use of the particles is that these are in any case not infectious, wherein in many cases also the UV radiation for inactivation of the RNA can be omitted.

In comparison to the use of inactivated CVB3 particles in lipofection as described by Kern et al. (loc. cit.), it was, first of all, unexpected that also the derived peptides and particles resulted in a reinforcement of the transfection. It was even shown that the efficiency is higher than for inactivated CVB3.

According to the invention, a method for creating such a particle comprises the steps of:

- Isolating native CV particles, preferably CVB particles;

- Heating the isolated CV particles, preferably CVB particles, for 5 - 20 min, preferably approximately for 10 min, to over 45°C, preferably approximately to 51°C.

Experiments performed in the laboratory of the inventors have shown that CV-A particles are created in a simple way by this method, in which the virus protein 4 was cleaved off.

Alternatively, CV-A particles can also be created using the following method:

- Providing a matrix of coupled CV receptor;

- Loading the matrix with native CVB3 particles; and

- Eluting the matrix.

It is here made use of the fact that virus protein 4 is cleaved off after interaction of the CV with the cellular receptor, so that only the remaining CV-A particle is internalized which is not infectious and according to the finding of the inventors acts endosomolytically.

In view of the above, the present invention relates further to a method for transfection of cells, preferably of cardiac myocytes, with a polyanion, preferably a therapeutic gene sequence, in which method at least one particle or peptide of the kind mentioned above is used in order to enhance the transfection, wherein preferably prior to the transfection a complex comprising the polyanion, the peptide and a cationic lipid, preferably DOTMA, is formed.

While the particles and peptides mentioned act generally endosomolytically, i.e. provide a high efficiency of the new method, there is a considerable advantage for lipofection of cardiac myocytes. The inventors have recognized, namely, that endosomolysis can comprise organo-specific aspects, so that the use of particles and peptides that are derived from CV, preferably from CVB3, is the method of choice for gene transfer at the heart muscle. It has turned out that other viruses are by far not as efficient, in particular, adeno viruses are not able to colonise this organ as efficiently as e.g. the cardiotrophic CVB3.

The transfected polyanion can be a DNA, an RNA or a correspondingly charged protein with a therapeutic effect.

In view of the above, the invention relates further to a method for formulating complexes for the transfer of polyanions, preferably of a therapeutic gene sequence into cells, preferably into cardiac myocytes, in which method the polyanion is incubated with at least one peptide or particle of the kind mentioned above.

In this connection, it is preferred if prior to the incubation with the polyanion the peptide is pre-incubated with a cationic lipid, preferably with DOTMA.

This method can be carried out in a particularly efficient, inexpensive and simple manner, the individual agents are merely to be mixed with each other one after the other and to be incubated for a short time, whereby the desired complex is formed spontaneously.

Thus, such complexes are vehicles that can be manufactured in a simple and, thus, inexpensive way, are immunologically safe and are used for the transfer of diagnostically, therapeutically or prophylactically acting agents into cells in particular of the cardiovascular system.

In view of the above, the present invention also relates to the use of a particle or a peptide of the kind mentioned above for therapy, diagnostics or prophylaxis of cardiovascular diseases, in particular of cardiomyopathies.

Furthermore, the invention relates to the use of a particle or a peptide of the kind mentioned above as an agent for improving

the transfection of cells, preferably of cardiac myocytes, in particular for improving lipofection in gene therapy.

The peptides and particles found cannot only be used in the scientific range or in individual preparation, rather, they are also suitable for the general use even in less specialized hospitals or in doctor's practices.

In view of the above, the present invention also relates to a therapeutic composition comprising a peptide or a particle of the kind mentioned above, as well as a kit for formulating transfectable complexes, in particular for gene therapy, comprising at least one particle or one peptide of the kind mentioned above.

Such therapeutic compositions and kits can comprise the particles and/or peptides in stock solutions or already in final concentrations, wherein, moreover, the reagents further required, such as the liposome formulation, may be available in the kit.

The peptides and particles can be generated, on the one hand, enzymatically by digestion of virus particles and, on the other hand, by genetic engineering.

In view of the above, the invention further relates to a replicable expression vector comprising a gene sequence encoding a peptide or a particle of the kind mentioned above in an expressible manner.

Further, the invention relates to a DNA isolate comprising a DNA sequence encoding a peptide or a particle of the kind mentioned above.

In this way, it is possible to produce the peptides and particles in large scale by microbiological methods or in-vitro-translation.

If by genetic manipulation replication incompetent virus particles are used, a very high safety is reached, since these particles can only be replicated in a helper cell that provides the destroyed functions *in trans*. These particles can then also be used for lipofection without further inactivation by UV radiation or thermal treatment.

Generating replication incompetent virus particles can be performed e.g. by deletion of coding genome sequences, preferably of the VP2 and the VP3 region which comprise the information for the replication relevant proteins. On the other hand, these replication relevant gene functions may also be destroyed by point mutations. The deleted sequences are replaced by other sequences, preferably non-coding sequences, in order to re-establish the optimal genome size for packing. According to the invention, the deleted or mutated genome sequences are then supplied in helper cells.

In view of the above, the present invention also relates to a vector plasmid having at least one DNA sequence encoding a genetically modified CV genome, preferably a CVB genome, more preferably a CVB3 genome, in which parts of the coding sequence are exchanged or modified, so that the virus genome is not in-

fectious, and comprising a promoter upstream of said DNA sequence. Further, the invention relates to a helper construct for complementing the parts of the coding sequence of the virus genome that have been modified or exchanged in the vector plasmid.

This helper construct can be a helper plasmid, a viral vector or a helper cell that is stably transfected with helper DNA encoding at least one of the exchanged or modified sequences.

For producing virus particles replication incompetent by genetic manipulation, host cells are transfected with the vector plasmid, wherein the exchanged or modified sequences in the host cell are complemented by the helper construct.

In that manner, replication incompetent virus particles can be simply and reproducibly reliably created which act endosomolytically within the scope of transfection of cells or, respectively, reinforce endocytosis.

Further features and advantages of the invention can be taken from the following description of preferred embodiments.

Example 1: CVB3 genome and CVB3 cDNA

Coxsackie viruses are representations of the genus entero viruses in the family of picorna viruses. Under natural conditions, Coxsackie viruses cause diseases merely in humans, however, the initial isolation of Coxsackie viruses is managed best in new-born mice which also serve for differentiation of viruses into two groups:

Group A comprising 23 serotypes and group B comprising 6 serotypes.

CVB, particularly CVB3, are considered to be frequent causative agents of viral heart muscle diseases which can appear both in this acute form and in chronic disease course. Myocarditis is often fatal for babies.

Like all picorna viruses, Coxsackie viruses also have icosahedral nucleo capsides, which consist of four virus proteins VP1, VP2, VP3 and VP4. While proteins VP1, VP2 and VP3 form the outer coat, VP4 is located at the inner side of the particles and associated with the single-stranded RNA genome. The genome is infectious per se; if it is incorporated in a cell under suitable conditions, the purified RNA can already induce an infection, since it possesses positive-sense orientation, so that the virus proteins can be translated from RNA without an intermediate step. The 3' end of the genomic RNA is polyadenylated, a small, virus-encoded protein V_{Pg} is covalently bound to the 5' end.

A schematic example for the CVB3 genome is shown in Fig. 1. The genome comprises a single, open reading frame which encodes a precursor protein. Already during its synthesis, this polyprotein is protelytically cleaved into the different viral components.

In the indicated manner, capsid proteins VP1-VP4 already mentioned emerge from the polyprotein from regions 1A through 1D and Vpg emerges from region 3B. Regions 2A and 3C code for proteases which cleave the polyprotein. The proteins emerging from

regions 2B and 2C are in connection with the host specificity of the viruses.

Region 3D codes for a DNA-dependent RNA-polymerase which performs the replication of the RNA genome in the host cell.

At the 5' and 3' end the genome comprises regions that are not yet translated (NTR), with the NTR region at the 5' end comprising a distinct secondary structure and allowing the binding of ribosomes, i.e. allowing the translation of the genome into the polyprotein.

The complete nucleotide sequence of a cDNA of an infectious CVB3 variant with a distinct tropism towards the heart is described in Klump et al. (loc. cit.). This infectious cDNA of CVB3 is available in the construct pCB3/T7 from Klump et al. or in the construct pcMV-CVB3 newly provided by the inventors. Upstream of the 5' end, a promoter (prom) is located which allows transcription of the cDNA into the RNA genome.

Example 2: Purification of CVB3 particles

Cell cultures having confluently grown cardio myocytes are infected with 1 - 5 infectious units (PFU) CVB3. After some hours, the cytopathic effect can be recognized.

After 6 - 12 hours, the cell culture supernatants as well as the cells are harvested.

The viruses are released from the cells by the usual freeze/melt lysis. This virus suspension is, first of all, purified from cell debris by centrifugation at 1,500 rpm.

The virus-comprising supernatant is applied onto 10 ml of a 5 - 30 % saccharose gradient and ultracentrifuged. In that way, a separation of native virions (sedimentation coefficient 160 S), CVB3-A particles (135 S) and proviruses (125 S) is reached. Proviruses are incompletely matured viruses which are present in large amounts in each preparation and are characterized by not yet performed cleavage of the VP0 protein into VP2 and VP4.

A-particles arise from native virions by cleavage of virus protein 4, a process naturally occurring in the infection. Then, they are not infectious any more, however, they can still be propagated in a cell.

The infectious particles that by experience can be found in the first five fractions are analysed by Western-Blot-analysis for the presence of viral particles.

For eliminating saccharose from the virus preparations, the relevant single fractions are dialyzed against PBS/20 mM $MgCl_2$ for 24 hours at 4°C.

The purified virus stocks are stored at -20°C. Dilutions of these virus stocks are seeded on host cells in order to check the virus titer in a usual plaque test.

In order to inactivate the CVB3 viruses, the virus stock is filled into a Petri dish and exposed to UV-C radiation for 30 - 60 minutes. During this process, the nucleic acids of the viruses are damaged, but not the biologically active proteins.

Example 3: Generation of CVB3-A particles

In the fractions generated according to example 2, only small amounts of CVB3-A particles can be found, so that these can be produced from infectious CVB3 particles in the following two manners:

A) native virions are heated at 51°C for 10 min and, thereby, change quantitatively into CVB3-A particles.

Thus, a method is provided for generating any desired amount of these particles that are otherwise released only in limiting amounts by infected cells.

B) The alternative is based on a column purification, in which recombinant CVB3 receptors are coupled to a matrix. The column is then loaded with active CVB3 particles which interact with the receptor and, in doing so, lose virus protein 4. By eluting the column the CVB3-A particles can be recovered.

Example 4: Endosomolytical activity

It could be shown that the CVB3 reinforced lipofection is receptor independent, so that no A-particles are generated during lipofection. A-particles are, however, particles naturally

arising from receptor-dependent infection and show higher endosomolytical activity than native virions. Thus, the results that have been gained with lipofection reinforced by A-particles are better than lipofection with native virions described by Kern et al.

The explicit endosomolytical activity of CVB3 could be shown by counterbalancing CVB3 mediated reinforcement of the lipofection with Bafilomycin, a specific inhibitor of the endosomal proton pump (Bowman et al., PNAS, volume 85, pages 7972-7976, 1988; Drose et al., Biochemistry, volume 32, pages 3902-3906, 1993). The infection of cells with CVB3, however, cannot be inhibited by Bafilomycin.

Peptides from the capsid region of CVB3 which are endosomolytically active are shown in the following table:

Table 1: CVB3 peptides having endosomolytical activity. Hydrophilic acidic or basic amino acids are in bold.

Peptide Sequence	Capsid region
SEQ ID No. 1 RVVYNAGMGVGNLTIF	VP2
SEQ ID No. 2 LPTMNTPGSCQFLTSDD	VP3
SEQ ID No. 3 KLTFMFCGSAMATG	VP3
SEQ ID No. 4 LTFMFCGSAMATGK	VP3
SEQ ID No. 5 KFLLAYSPPGAGAP	VP3
SEQ ID No. 6 FLLAYSPPGAGAPTKRVD	VP3
SEQ ID No. 7 ILTHQIMYVPPGGPVDKVD	VP1
SEQ ID No. 8 DKVDSYVWQTSTNPSVFW	VP1
SEQ ID No. 9 VYGINTLNNMGTLYARH	VP1
SEQ ID No. 10 YGVWRDYLKDSEATAEDQ	VP2

The peptides may raise the problem that it is difficult to combine them with liposomes due to their amphipathic characteristics, since the liposomes will be destroyed thereby. This problem can be solved by the fact that some additional acidic amino acids are synthetized N-terminally or C-terminally and that the synthetic peptide is then bound via a positively charged polylysine-bridge to the DNA to be transferred. This has the further advantage that Transferrin-coupled polylysine may be used so that an efficient binding to the Transferrin-receptor is possible (Wagner, loc. cit.).

Example 5: Replication incompetent, genetically modified virus particles

Starting point for the production of genetically modified, replication incompetent virus particles is a vector plasmid as shown in Fig. 2. This vector plasmid comprises downstream of a promoter a DNA-sequence encoding the RNA-genome shown in Fig. 1, however, with certain parts of the RNA-genome being exchanged or modified e.g. by point mutations in such a way that the RNA-genome itself is not infectious any more.

In Fig. 2, the RNA-genome shown in Fig. 1 is schematically divided into a first part I as well as a second part II. Parts I and II need not necessarily be schematically arranged in a row, they can also be present in any sequence and in multiple form.

Part I represents the exchanged or modified sequence part, whereas part II represents the residual genome from Fig. 1.

The vector plasmids shown in Fig. 2 are then co-transfected into host cells by means of helper constructs in order to create replication incompetent virus particles. The helper constructs must complement exchanged or modified part I in order to make this co-transfection successful.

This can be done, on the one hand, by amplifying part I by means of specific PCR-primers from plasmid pCMV-CVB3 mentioned in example 1. These amplificates can be introduced into host cells by means of a viral vector, wherein, on the other hand, a helper cell can also be stably transfected with these amplificates. Thus, the helper cells serve as host cells in the transfection with vector plasmid and supply the missing or modified parts of the virus genomes *in trans*.

It is preferred, however, if the helper constructs are also plasmids which are stably or transiently transfected into host cells in order to be then transcribed into RNA which, again, is translatable in order to create structural and non-structural proteins, for which the vector plasmid itself is not coding.

To this end, it is required to clone e.g. into the pCR-script™-plasmid a promoter, e.g. the CMV-promoter, and an IRES (internal ribosomal entry site). Downstream of the promoter, the amplificates are inserted comprising parts I of CVB3. By means of IRES, the translation efficiency of the helper constructs is increased, for example, the IRES from EMVC (Encyphalomyocarditis virus), the EMCV-IRES from CLONETECH may be used.

Thus, helper plasmids are generated which can be reinforced in bacteria and which can be transcribed into RNA which, again, can be translated in order to complement the translation products of the vector plasmids such that replication incompetent virus particles are formed.

To this end, the helper cells which are transiently or stably transfected with the helper plasmid are transfected with a suitable vector plasmid which is then complemented by the host cell *in trans*.

In that way, replication incompetent virus particles are formed that can be used after suitable purification.

A way that allows a higher variability is co-transforming host cells with the vector plasmid shown in Fig. 2 and the suitable, complementing helper plasmid shown in Fig. 3, whereby the replication incompetent virus particles are formed.

Example 6: CVB3-reinforced lipofection

Chinese-Hamster-Ovarian cells (CHO), rat myoblast cells (H9C2), human cervix-carcinoma cells (HeLa), primary human fibroblasts as well as primary adult heart muscle cells of the pig were used as target cells.

On the first day, 5 x 10^4 cells were seeded onto 24-holes-plates. On the second day, the following solutions were prepared:

Solution A: 150 µl serum-free medium
5 µl Lipofectin (GIBCO/BRL)
1 particle per cell CVB3
or 100 ng-10 µg peptide solution, respectively

Solution A is incubated at room temperature for 30 min.

Solution B: 1.5 µg plasmid-DNA (e.g. pCMVLacZ)
ad 150 µl serum-free medium

Solutions A and B are mixed and incubated at room temperature for 15 min.

Prior to adding the completed lipofection solution, the cells are washed once with serum-free medium.

The medium is completely removed and 300 µl lipofection solution is applied onto the 24-holes-plate. Thereafter, the cells are incubated at 5 % CO_2 and 37°C for 5 hours. After that time, the lipofection solution is removed and replaced by cell culture medium.

The evaluation of the transfection is performed on the next day using an In-situ-Lac-Z-assay:

Transfected plasmid pCMVLacZ is coding for the β-galactosidase which generates from the solution that first is colorless a blue, insoluble reaction product in the cells. The cells are thereby first fixated and then incubated with the coloring solution. The transfection efficiency is calculated from the num-

ber of dyed cells/total number of cells and is determined in the microscope.

First results obtained in the laboratory of the inventors show that transfection can be mediated by the particles according to example 2, 3 and 5 as well as by the peptides according to example 4, the efficiency of which is above the efficiency of adeno virus-mediated lipofection.

SEQUENCE LISTING

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<120>Use of Coxsackie virus for improving the transfection of cells

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Asp Gln

Claims

1. Non-infectious particles or peptides derived from the Coxsackie virus, preferably derived from CVB, more preferably derived from CVB3, which are suitable to act endosomolytically within the scope of a transfection of cells.

2. Non-infectious particles or peptides derived from the Coxsackie virus, preferably derived from CVB, more preferably derived from CVB3, which are suitable to enhance endocytosis within the scope of a transfection of cells.

3. Particles of claim 1 or 2, selected from group:

   a) empty CV capsid particles, preferably CVB capsid particles;

   b) CV particles, preferably CVB-A particles, generated by cleaving off virus protein 4 (VP4);

   c) CV particles, preferably CVB provirus particles, in which the virus proteins 2 (VP2) and 4 (VP4) are still fused;

   d) modified forms of particles from a), b) or c); and

   e) CV particles, preferably CVB particles, more preferably CVB3 particles, that are replication-incompetent by genetic modification.

4. A peptide of claim 1 or 2, preferably comprising less than 30 amino acids and being selected from group:

a) Peptides with the amino acid sequences SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 5, SEQ ID No. 6, SEQ ID No. 7, SEQ ID No. 8, SEQ ID No. 9, SEQ ID No. 10 from the sequence listing;

b) Peptides, in which in comparison with peptides from a) one or more of the amino acids are missing or exchanged against another; and

c) Peptides or polypeptides which comprise the amino acid sequence of one of the peptides from a) or b).

5. A method for generating a particle of claim 3, comprising the steps:

- Isolating native Coxsackie virus particles;

- Heating the isolated Coxsackie virus particles for 5 - 20 min, preferably approximately 10 min to over 45°C, preferably approximately to 51°C.

6. A method for generating a particle of claim 3, comprising the steps:

- Providing a matrix of coupled Coxsackie virus receptor;

- Loading the matrix with native Coxsackie virus particles; and

- Eluting the matrix.

7. A method for transfection of cells, preferably of cardiac myocytes, with a polyanion, preferably a therapeutic gene sequence, in which method at least one particle or peptide of any of claims 1 through 4 is used in order to enhance transfection.

8. The method of claim 7, characterized in that prior to transfection a complex comprising said polyanion, peptide and cationic lipid, preferably DOTMA, is formed.

9. A method for formulating complexes for the transfer of a polyanion, preferably a therapeutic gene sequence, into cells, preferably into cardiac myocytes, in which method the polyanion is incubated with at least one particle or peptide of one of claims 1 through 4.

10. The method of claim 9, characterized in that the peptide is pre-incubated with a cationic lipid, preferably with DOTMA, prior to the incubation with said polyanion.

11. The use of a particle or a peptide according to any of claims 1 through 4, for therapy, diagnostics or prophylaxis of cardiovascular diseases, in particular of cardiomyopathies.

12. The use of a particle or a peptide according to any of claims 1 through 4 as an agent for improving transfection of cells, preferably cardiac myocytes, in particular for improving lipofection in gene therapy.

13. A replicable expression vector comprising a gene sequence encoding a peptide or a particle according to any of claims 1 through 4 in an expressible manner.

14. A DNA isolate comprising a DNA sequence encoding a particle or a peptide according to any of claims 1 through 4.

15. A kit for formulating transfectable complexes, in particular for gene therapy, comprising at least one particle or one peptide according to any of claims 1 through 4.

16. A therapeutic composition, comprising at least one particle or a peptide according to any of claims 1 through 4.

17. A vector plasmid, comprising at least one DNA sequence encoding a genetically modified CV genome, preferably a CVB genome, more preferably a CVB3 genome, in which parts of the coding sequence are exchanged or modified, so that the virus genome is not infectious, and comprising a promoter upstream of said DNA sequence.

18. A helper construct for complementing the parts of the coding sequence of said virus genome that have been modified or exchanged in the vector plasmid of claim 17.

19. The helper construct of claim 18, characterized in that it is a helper plasmid encoding at least one of said exchanged or modified parts in a translatable manner.

20. The helper construct of claim 18, characterized in that it is a viral vector encoding at least one of said exchanged or modified parts in a translatable manner.

21. The helper construct of claim 18, characterized in that it is a helper cell that is stably transfected with helper DNA encoding at least one of said exchanged or modified parts.

22. A method for generating said particle of claim 3e) that is replication-incompetent by genetic modification, comprising the steps:

- Transfecting host cells with the vector plasmid of claim 17; and

- Complementing said exchanged or modified parts in the host cell by the helper construct according to any of claims 18 through 20.

23. The method of claim 22, characterized in that said host cell is the helper cell of claim 21.

PCT

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INTERNATIONALE ANMELDUNG VERÖFFENTLICHT NACH DEM VERTRAG ÜBER DIE INTERNATIONALE ZUSAMMENARBEIT AUF DEM GEBIET DES PATENTWESENS (PCT)

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Vor Ablauf der für Änderungen der Ansprüche zugelassenen Frist; Veröffentlichung wird wiederholt falls Änderungen eintreffen.

(54) Title: USE OF COXSACKIE VIRUSES FOR IMPROVING CELL TRANSFECTION

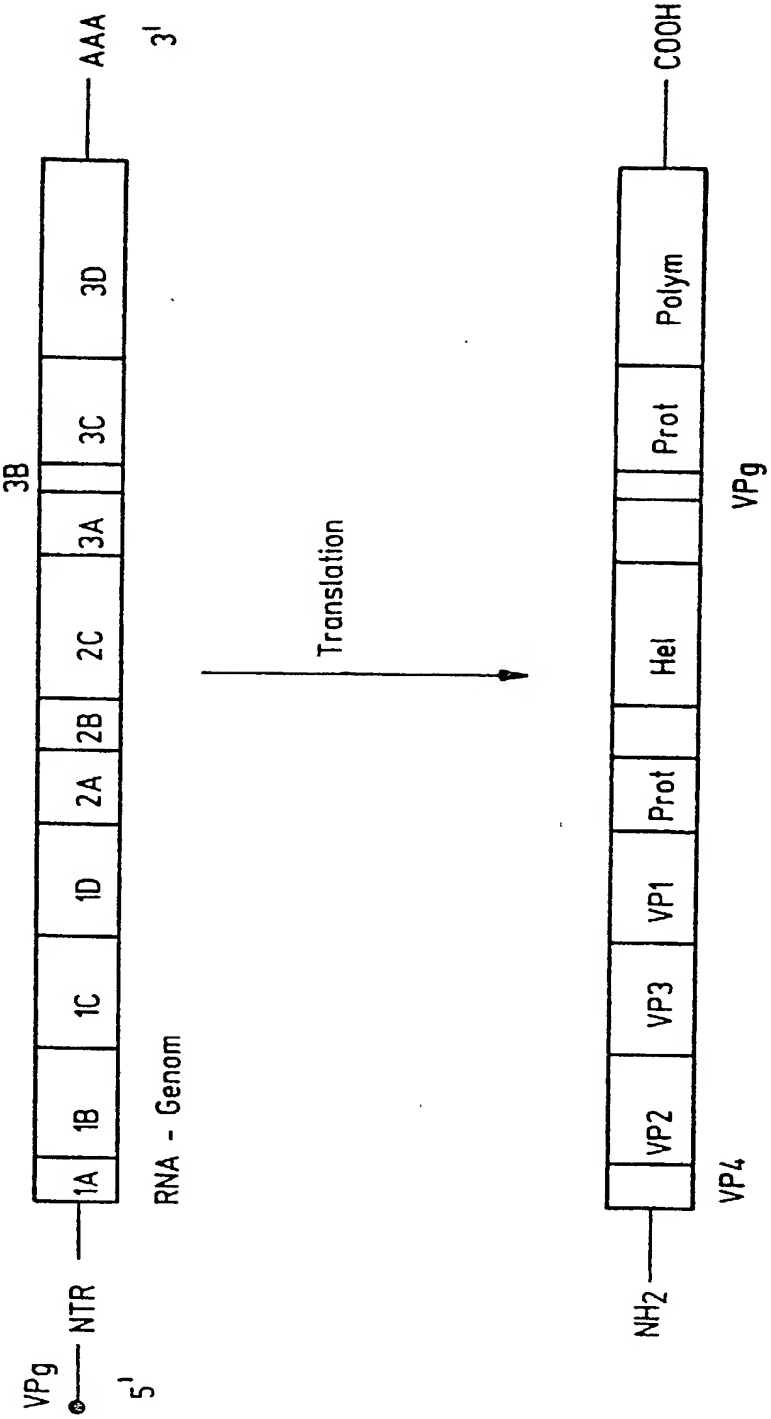
(54) Bezeichnung: VERWENDUNG VON COXSACKIEVIREN ZUR VERBESSERUNG DER TRANSFEKTION VON ZELLEN

(57) Abstract

The invention relates to non-infectious particles or peptides derived from the Coxsackie virus which are suited to endosomolytic action and/or endocytose reinforcement in a cell transfection.

(57) Zusammenfassung

Es werden von dem Coxsackievirus abgeleitete, nicht infektiöse Partikel oder Peptide beschrieben, die dazu geeignet sind, im Rahmen einer Transfektion von Zellen endosomolytisch zu wirken und/oder die Endocytose zu verstärken.


VPg
NTR
5'
1A
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1C
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2A
2B
2C
3A
3B
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3D
AAA
3'
RNA - Genom
Translation
NH2
VP2
VP3
VP1
Prot
Hel
Prot
Polym
COOH
VP4
VPg
Polyprotein

Fig.1

Fig.2

Fig.3

DECLARATION - USA PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name;

I believe I am an original, first and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled USE OF COXSACKIE VIRUSES FOR IMPROVING TRANSFECTION OF CELLS; this specification is the U.S. National Phase under 35 U.S.C. § 371 of International Application No. PCT/EP00/03588, which was filed on April 20, 2000. The Application for Letters Patent in the United States was mailed on October 23, 2001 and assigned U.S. Serial No. 10/009,040.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above;

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56;

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)-(d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

PRIOR FOREIGN APPLICATION			Priority Claimed
No.: **19918446.1**	Country: **Germany**	Date Filed: **April 23, 1999**	**YES**

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful, false statements may jeopardize the validity of the application or any patent issued thereon.

1-00

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3-00

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